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Wound Healing and Collagen Thermal Damage in 7.5-μsec Pulsed CO_2 Laser Skin Incisions

David L. Sanders, BE and Lou Reinisch, PhD*

Department of Otolaryngology, Vanderbilt Bill Wilkerson Center for Otolaryngology and Communication Sciences, Vanderbilt University Medical Center, Nashville, Tennessee 37232

Background and Objective: **Wound-healing delays caused by lateral thermal damage to tissue remain a drawback of CO_2 surgical lasers. This study compares the thermal damage and wound-healing properties of a 7.5-μs pulsed CO_2 laser with scalpel and continuous wave (CW) CO_2 laser incisions.**
Study Design/Materials and Methods: **We created incisions on the dorsal pelts of rats with a 7.5-μs pulsed CO_2 laser at 5-, 10-, or 15-Hz repetition rate, a conventional CW laser, or scalpel. Animals were euthanized at postoperative days 3, 7, 14, 21, and 80. Tissue was harvested and analyzed histologically and for wound tensile strength. In addition, tissue was harvested acutely and analyzed for acute thermal injury lateral to the incisions.**
Results: **Incisions made with the pulsed laser had significantly higher tensile strength and histologic rankings than did CW laser incisions at days 3–21, producing 118 μm of thermal damage to tissue as compared with 333 μm for CW laser. Pulsed laser incisions were not statistically different than scalpel incisions at days 3–14 of healing. Mathematical modeling showed the pulsed laser to produce a wound healing delay of 1.0 day by tensiometry and 1.9 days by histology, compared with 3.2 days by tensiometry and 6.0 days by histology for CW laser. There were no significant differences in wound healing when the pulsed laser was used at repetition rates of 5–15 Hz.**
Conclusions: **Using a 7.5-μs pulse duration, CO_2 laser incisions healed at a rate similar to scalpel incisions and reduced the wound-healing delay seen with typical surgical CO_2 lasers. Lasers Surg. Med. 26:22–32, 2000. © 2000 Wiley-Liss, Inc.**

Key words: **histology; laser surgery; pulse structure; Sprague-Dawley rat; tensiometry**

INTRODUCTION

The CO_2 laser is commonly used in a variety of surgical procedures and has been proven to be a versatile tool in many surgical fields. The laser affords the advantages of precise, hemostatic incisions that can be made where a scalpel would have limited or restricted use. Because of these properties, lasers have been viewed as an alternative to the scalpel, but lasers also offer a variety of unique applications, such as skin resurfacing and scar revision.

Despite these advantages, the CO_2 laser and lasers in general have the disadvantage of causing a delay in wound healing when compared with scalpel incisions [1–3]. This is secondary to thermal damage to tissues surrounding the incisional area and thus produces tissue that must be removed before wounds can be repaired [4–6]. This delay has been observed through the first 30 days of wound healing [3,7]. The CO_2 laser emits light

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*Correspondence to: Lou Reinisch, PhD, S-2100 Medical Center North, Vanderbilt University Medical Center, Nashville, TN 37232-2559. E-mail: reinisl@ctrvax.vanderbilt.edu

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at a wavelength of 10.6 μm, which is absorbed strongly by tissues with a high water content. This water absorbs light energy and is heated and vaporizes tissue, allowing the laser to cut. During this process, however, some of this thermal energy is conducted to surrounding tissues and can damage the cellular and extracellular structures. In addition, some of the tissue at the perimeter of the laser beam is exposed to low levels of laser energy, and this subablative energy also damages surrounding tissue. This excess thermal damage can be observed on the border of tissues lateral to a laser incision. The thickness of this thermal damage has been correlated with the delay in wound healing [8,9].

Researchers have attempted to modify the CO_2 laser to minimize this thermal injury and thus reduce wound-healing delays. A variety of methods have been studied, including the use of a reduced spot size [10], microspot micromanipulators for precise beam delivery, computer-controlled beam delivery [5,11], and modification to the energy pulse duration and repetition rate. Some improvements have been gained through these techniques, but the healing delay remains significant.

The laser pulse structure and duration have been shown to be important parameters in minimizing thermal damage. Walsh et al. showed that CO_2 laser with short pulse structures cause less thermal damage than those created by a continuous wave (CW) laser [12], and this has been confirmed by others [6,13–14]. These studies have identified the importance of the pulse length in relation to the thermal relaxation time, a time constant that is the amount of time required for thermal energy to diffuse to surrounding tissues, thus heating and injuring them. This time has been estimated by several models as ranging from 7 to 700 μsec [12,15–16]. Thus, a pulse duration shorter than this should show less thermal damage because the laser energy is delivered so quickly that it cannot diffuse to surrounding tissues. Studies in canine oral mucosa have shown decreases in wound-healing delays when using a 60–100-μsec pulse duration [13]. Another parameter that influences excess thermal damage is the thermal recovery time. It is the time required for heat to dissipate after a laser pulse is delivered. It represents a limit to how frequently the laser pulses can be repeated without heat buildup in the tissue and an increase in thermal damage. This has been estimated to range from 200 to 300 msec in skin [12]; thus, repetition rates greater than approximately 5 Hz can show excess thermal damage. However, another model indicates this thermal accumulation can occur at rates faster than 0.1 Hz [15].

The purpose of the present study was to investigate the wound-healing properties of a CO_2 laser with a short pulse duration. A laser with a 7.5 μsec pulse duration was used on rat dermis to determine whether it would decrease thermal damage lateral to the laser incision and thus minimize or even eliminate the delay in wound healing as compared with CW and scalpel incisions. By developing lasers that have more favorable wound-healing properties, the major disadvantage of laser incisions could be minimized and nevertheless offer the unique advantages that the CO_2 laser affords for surgery.

MATERIALS AND METHODS

All studies were approved by the Vanderbilt University Animal Care Committee and the Animal Use Subcommittee.

The rat model has been previously validated for the study of skin-incision healing, including laser incisions [5,17]. The present study used male Sprague-Dawley rats that were initially 250 g in weight. Thirty-six animals were used, and these were divided into five groups so that wound healing over time could be studied. Two animals were assigned to the acute time group, eight each to the 3-day, 7-day, 14-day, and 21-day groups, and the remaining two animals to the 80-day group. Each animal was large enough to allow six incisions on its dorsal pelt. Incisions were created by one of three methods. The first method used a #10 scalpel blade, with direct pressure with cotton gauze to obtain hemostasis. The second method used a Sharplan 1060 CW CO_2 laser (Sharplan Lasers, Inc., Allendale, NJ), with the parameters of 5 W, 0.2-sec pulse duration, and frequency of 1 Hz. This laser was delivered by articulated arm and focused to a 0.2-mm spot size with a Gaussian (TEM_{oo}) beam profile. The third method used a 7.5-μsec pulse width sealed TEA CO_2 laser (Argus Photonics Group, Jupiter, FL) delivered at either 5 Hz, 10 Hz, or 15 Hz repetition rate. This laser delivers a 30–60 mJ pulse energy with a Gaussian (TEM_{oo}) beam profile and a focused 0.2-mm spot size using the same handpiece for both lasers.

Animals were anesthetized with inhaled Metofane vapor (Methxoyflurane, Pitman-Moore, Inc., Mundelein, IL) until there was no response to foot-pad pinching. At this onset of anesthesia,

the dorsal pelt was shaved, and the animals were placed in the operating field. Two small incisions were made with a scalpel just below the neckline, each 2 cm lateral to the midline. A 10-cm scalpel guide was placed into this incision and inserted, parallel to the midline, along the fascial plane between skin and muscle layers. Approximately 2.0-cm full-thickness longitudinal skin incisions were made above the scalpel guide by one of the previously described methods. These were spaced along the skin 1.0 cm apart. Three incisions were made along each scalpel guide, for six incisions per animal. These six incisions consisted of one to two scalpel incisions, one to two made with the continuous beam laser, and three pulsed laser incisions (one at each of the three repetition rates). After completion, the scalpel guides were removed and the wounds cleaned with saline and cotton gauze to remove any charred tissue or dried blood. Animals in the acute time point group were killed at this point, and tissue samples were collected for histologic analysis using the techniques described below. All other animals were treated as follows. Each incision was closed primarily with two interrupted 4-0 monofiliament sutures. Antibiotic ointment (Polymyxin B Sulfate, Bacitracin Zinc, Neomycin Sulfate; Altaire Pharmaceuticals, Inc., Holbrook, NY) was then applied liberally to each incision and at the site of scalpel guide insertion. Anesthetic gas was withdrawn, and the rat was observed while it recovered. Animals were then housed in cages with food and water ad libitum until the designated time of death.

At the group's corresponding time point, each animal was euthanized with an intramuscular injection of thiopental. For each incision, sutures were cut and removed (if still present), and tissue biopsies were cut from the rat pelt. From each incision, two samples were obtained. The first was a 1.0- × 2.0-cm tissue strip that was placed in normal saline for tensiometric analysis. The second was a smaller section of the incision (approximately 4 mm) that was harvested for histologic examination. These were fixed in 10% formaldehyde solution, embedded in paraffin, and sectioned. Using standard procedures, tissue sections were stained with either hematoxylin and eosin or Masson's trichrome stains. For animals in the acute time group, only histologic samples were harvested.

Tensiometry was performed on 3-day, 7-day, 14-day, 21-day, and 80-day skin samples to measure the physical strength of healing incisions. Measurements were performed either on the day of harvest or the next day after storing the samples in saline-soaked gauze and freezing them overnight. The tissue strips (approximately 1.0 cm of incision) were positioned in the jaws of an Instron 5542 tensiometer (Instron, Canton, MA). Peak breaking forces were measured and converted to maximal tensile strength values by dividing the breaking force by the cross section of the tissue that broke. Tensile strength is measured as kilograms of force per square millimeter of incisional cross section. The skin thickness was assumed to be constant for all specimens. Values were recorded on a personal computer, stored in a spreadsheet, and averaged for each incision type at each time point. Statistical comparisons were made with analysis of variance (ANOVA) using a Bonferoni–Dunn correction.

At each time point, the histologic features of the healing incisions were examined at 40–100× magnification using a Zeiss Axioplan II light microscope (Carl Zeiss Inc., Thornwood, NY). These were evaluated in a blinded fashion and scored based on their histologic features. Only the features pertinent at each time point were used (at 3 days: acute inflammation, reepithelialization, and zone of thermal injury; at 7 days: reepithelialization, inflammation, granulation tissue formation, and collagen maturity; at 14 and 21 days: epithelialization, granulation tissue, and collagen maturity). Scoring was subjective and based on how each compared with other samples at the same time point. For each feature, every slide was graded 1 through 4, with higher numbers representing a greater degree of wound healing (less acute inflammation, more mature collagen, etc.). Values were averaged for each criteria for each of the five incision types at each time point. These scores were summed to produce a total histologic score for each incisional type at each time point. Statistical analysis at each time point was performed with the Mann–Whitney *U* nonparametric test to determine differences between incisional types.

For acute time point incisions, histologic slides were examined and morphometric analysis was performed. Images were digitized with a Carl Zeiss digital video camera (Model ZVS-3C75DE). Zeiss ImagePro version 3.0 software (Media Cybernetics, Silver Spring, MD) was used to outline the zone of thermal damage, as indicated by color changes on Masson's trichrome–stained tissue. The average thickness from this border to the wound edge was then calculated for each incision. Values were averaged for each incisional type and

compared using an ANOVA with Bonferoni–Dunn correction.

Mathematical modeling was employed to estimate the wound-healing delay as compared with that made with the scalpel. The Henderson-Hasselbach two-state equation was used to obtain a best-fit line for the data. Modeling was performed for both the tensiometric and histologic data. This model assumes a two-state situation in which one state is the wound at the time of incision and the second state is at the point of maximal healing (estimated by average tensile strength at day 80). The data were fit to the model with Mac Curve Fit version 1.4 software (Kevin Raner, Victoria, Australia).

RESULTS

Acute Thermal Damage

Figure 1 shows representative micrographs of tissue obtained acutely after laser incision. Acute thermal injury can be seen as a discoloration along the lateral border of each incision. The zone of thermal injury was greatest for incisions made with the 0.2-sec pulse CW CO_2 laser. The average depth of thermal damage was 0 μm for the scalpel incisions, 330 μm for the CW CO_2 laser incisions, 120 μm for the pulsed CO_2 laser at 5 Hz, and 152 μm for the pulsed CO_2 laser at 15 Hz. The average thermal damage for the pulsed CO_2 laser at 5, 10, and 15 Hz repetition rate was 118 μm. Figure 2 shows the average thermal injury thickness for each type of laser incision. The "pulsed" category represents the average of the three different pulsed laser repetition rates (N = 35). An ANOVA comparison with Bonferonni–Dunn correction showed that there was a significant decrease in thermal injury between the CW laser and each of the pulsed laser groups ($P < 0.001$). In addition, there was a significant difference between the 5-Hz and 10-Hz pulsed laser groups ($P = 0.01$) and between the 10-Hz and 15-Hz pulsed laser groups ($P = 0.01$).

Tensiometry

Full-thickness skin sample strength was determined at each time point with a tensiometer. The incisions made with a CW CO_2 laser were consistently the weakest, whereas those made with the pulsed CO_2 laser were nearly as strong as the scalpel incisions. These average tensile strengths are plotted in Figure 3. Asterisks mark values that are significantly weaker than scalpel incisions at the same time point ($P \leq 0.05$, ANOVA with Bonferonni–Dunn correction). CW laser incisions were 49% as strong as scalpel incisions at 3 days, 38% at 7 days, 47% at 14 days, and 74% at 21 days of healing. Only at 80 days were the CW incisions statistically as strong as either of the other techniques. There was a slight trend toward stronger scalpel incisions at days 3–21. Pulsed laser incisions were 92% as strong as scalpel incisions at 3 days, 80% at 7 days, 96% at 14 days, and 88% at 21 days of healing. This difference reached statistical significance only at 21 days ($P = 0.046$). A comparison of the three repetition rates showed no statistically significant differences between them at any time point. There were no statistical differences between any of the incisional types at the 80-day time point.

Histology

Representative micrographs of healing incisions are shown in Figure 4. Overall, differences in wound healing were more apparent at the earlier time points. At day 3, scalpel incisions showed complete restoration of the epithelial barrier, little acute inflammatory cell reaction, and no thermal injury to surrounding tissue. The pulsed laser incisions showed complete reepithelialization in about 75% of samples, minimal or no residual thermal damage, and mild inflammatory response. The CW laser incisions, in contrast, had a large quantity of coagulum with incomplete restoration of the epithelial boundary, more uncleared thermally damaged collagen, and a much larger amount of acute inflammatory response.

At day 7, similar results were observed, although thermally damaged collagen was no longer visible in any of the incisions. Epithelialization was complete for approximately 50% of the CW laser samples and in all of the scalpel and pulsed laser wounds, with the epithelial layer maturity being greatest for the scalpel. Granulation tissue was visible, with scalpel incisions having the least area, with slightly more for pulsed incisions, and a large amount for CW incisions, indicative of the larger area of damage that required repair. New collagen maturity was assessed by examining collagen fiber bundle shape and a return to normal dark staining with trichrome stain. Scalpel incisions had the most mature collagen, with slightly less maturity for pulsed incisions, and the most immature collagen for CW laser wounds.

Results at day 14 were very similar to those at day 7. Epithelial boundaries were restored for

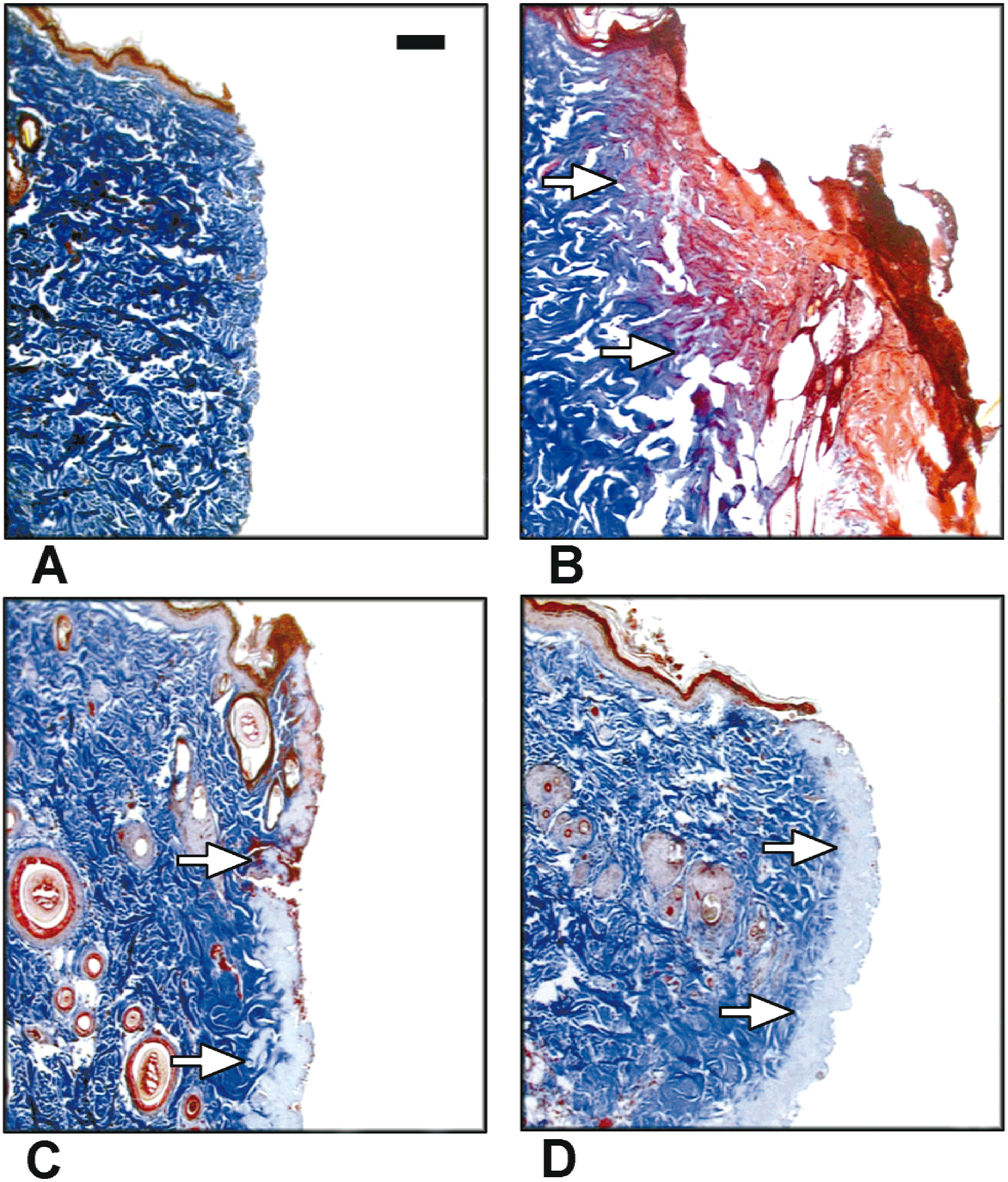

Fig. 1. Representative histology of tissue sections at the time of acute incision. Arrows indicate the lateral border of collagen thermal damage. **A:** Scalpel incision. **B:** Continuous wave laser with a 0.2-sec pulse length. **C:** 7.5-μsec pulsed laser at 5 Hz. **D:** 7.5-μsec pulsed laser at 15 Hz. Masson's trichrome stain. Scale bar = 100 μm.

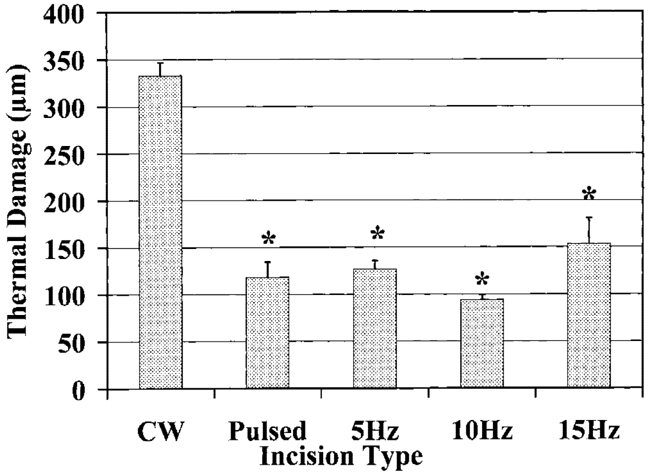

Fig. 2. Acute collagen thermal damage lateral to the incisional border (n = 20 at 5 Hz, 12 at 10 Hz, and 3 at 15 Hz). Pulsed category represents a mean value for the three pulsed laser repetition rates (n = 35). Error bars are standard errors of the means. Asterisks indicate a statistically significant decrease from continuous wave (CW) laser ($P < 0.001$).

all CW incisions at this time point, whereas those of scalpel and pulsed laser showed the most maturity and return to normal morphology. Collagen continued to mature for all groups. At day 21, there were fewer differences between the groups. CW laser incisions had maturing epithelial layers, whereas the others were nearly fully matured. CW laser still demonstrated the greatest amount of granulation tissue, with slightly less collagen maturity as compared with the other incisional types. Few differences were observed between the scalpel and pulsed laser incisions.

Histology at day 80 showed characteristics of well-healed incisions for all groups. Characteristic scar tissue was evident, with a slight increase in scar tissue seen with incisions created by CW laser. Scalpel and pulsed CO_2 laser incisions were similar at this time point.

Histologic scoring was performed to quantify the rate and degree of wound healing, with a total histologic score representing the sum of the individual criteria scores at each time point (as listed in Materials and Methods). Figure 5 shows the relative ranks of the histologic scores for the incision groups. These are normalized by the ranking for the scalpel group at each time point. At each time point, there is a significant difference between histologic ranks of the scalpel, pulsed laser, and CW laser ($P < 0.05$), except at day 21, when there was no statistical difference between CW and pulsed laser groups. However, no differences were found between individual pulsed laser repetition rate groups (5 Hz, 10 Hz, 15 Hz) at any time point ($P = 0.25$–0.59).

Wound-Healing Delay

An attempt was made to quantify the wound-healing delay for each laser incision type using mathematical modeling. As described in Materials and Methods, a two-state model was used to fit the tensiometric and histologic data. Figure 6 shows a plot of the tensiometric data, with the solid lines representing the two-state model estimation; 90 kg/mm^2, the average strength at day 80, was used in the model as the maximal tensile strength. The healing time to achieve maximal tensile half-strength was calculated for each curve and is represented by an arrow. The time to half-strength for scalpel incisions was 24.5 days ($R^2 = 0.99$). For pulsed laser incisions, this value was 25.5 days ($R^2 = 0.98$), representing a 1.0-day delay in wound healing. For the CW laser, this time was 27.8 days ($R^2 = 0.98$), or a 3.2-day delay as compared with scalpel. A similar method was used to estimate wound-healing delays from the histologic data. Because the scoring was performed only within each time point, each score was normalized by the corresponding score for scalpel incisions on that day. The wound-healing delay as compared with scalpel incision was calculated to be 1.9 days for pulsed CO_2 laser and 6.0 days for CW laser incisions.

DISCUSSION

Although the CO_2 laser has been proven to be a reliable tool for a variety of surgical procedures, it has the significant disadvantage of creating excess thermal damage while cutting. The benefits, such as being able to precisely create sterile, hemostatic incisions remotely has been tempered by the result of this thermal damage, delays in wound healing, and other adverse effects such as increased erythema, scarring, and edema [16,18–19]. Over the approximately 30 years the CO_2 laser has been used as a surgical tool, a variety of techniques have been studied or developed to minimize this thermal injury to surrounding tissues. The laser pulse duration has been presented as one factor that is important, and lasers with short pulse structure have been shown to produce less thermal damage than standard shuttered CW lasers. This study attempts to correlate previous studies of acute thermal damage with actual rates of wound healing in the skin by using a rat model.

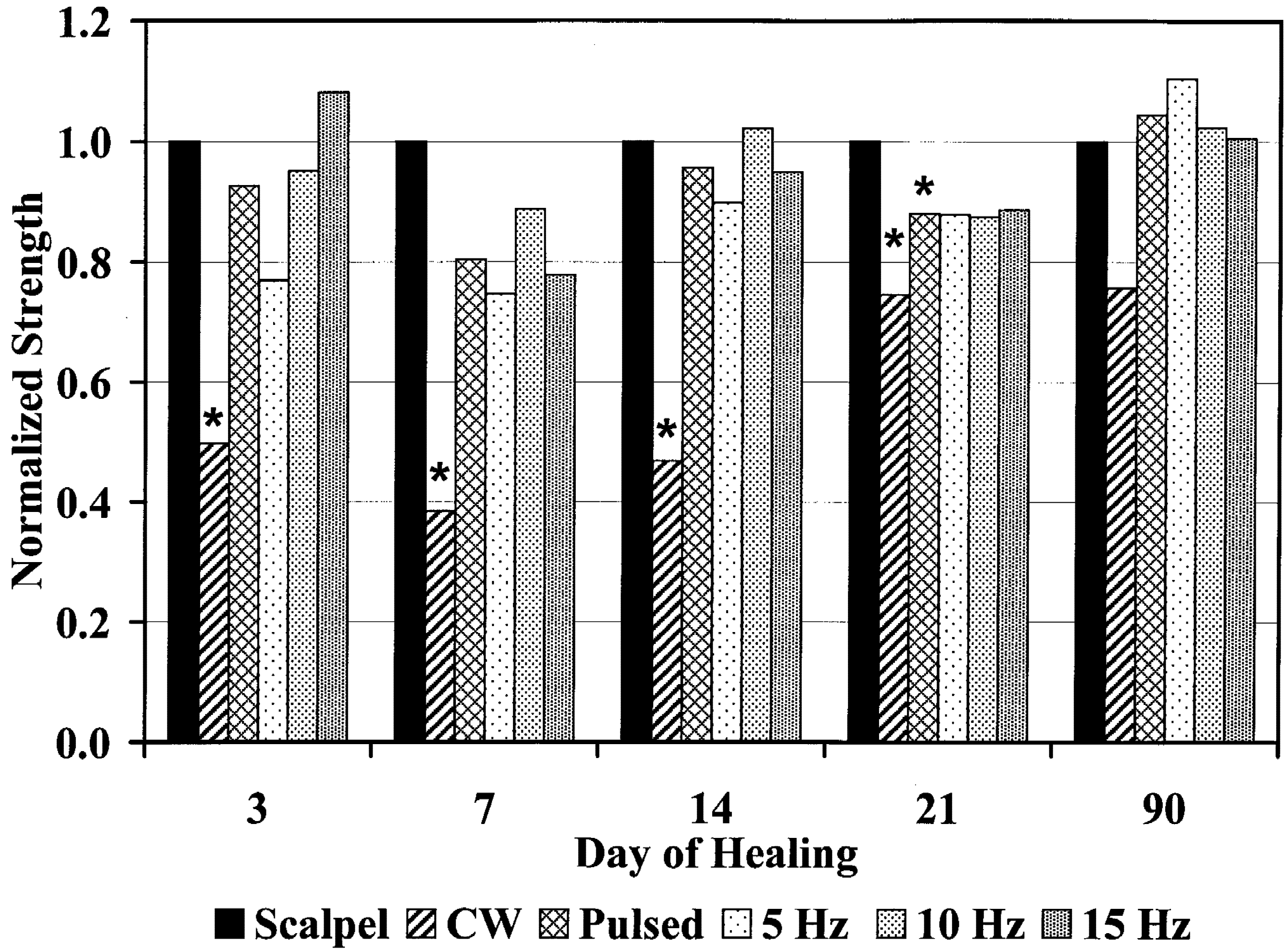

Fig. 3. Mean tensile strengths of healing incisions versus time. Values are normalized by the mean scalpel strength at each time point. Asterisks indicate a significant decrease in tensile strength as compared with scalpel at that time point ($P < 0.05$). Pulsed category represents a mean value for the three pulsed laser repetition rates. CW, continuous wave.

Studies have suggested that even shorter pulse lengths than those currently used clinically may be beneficial in reducing wound-healing delays [12–13,16]. This study investigates a short pulse length CO_2 laser (7.5 μsec) to compare wound-healing properties with a CW laser and scalpel. This pulsed laser was tested by using a standard surgical handpiece and was compared directly with the CW laser. Tissue cutting rates at 15 Hz were similar to the 5-W CW laser with 0.2-sec pulse duration in repeat pulse mode. The hemostatic properties were retained with this short pulse length but were somewhat reduced as compared with CW laser. Occasionally, direct pressure on the incision was required to achieve hemostasis with the pulsed laser. No objective measurements were made, but hemostasis was judged as adequate for these skin incisions.

Ideally, we would like to have laser pulses short enough to minimize lateral thermal damage. However, we do not want to use laser pulses that are too short because they will fail to provide hemostasis. Also, ultrashort pulses (≤10 nsec) cause additional complications, such as photoacoustic damage and nonlinear optical effects [20]. We did not observe any evidence of tissue disruption by photoacoustic pulses or cavitation. The pulse lengths of microseconds are probably long enough to minimize or eliminate acoustic and cavitation damages.

Thermal Injury

In measuring acute thermal injury, we found results similar to those of previous investigators when cutting with the CW laser [2,4,15]. We measured 333 μm of lateral thermal damage when operating the CW laser at the parameters described in Materials and Methods. The pulsed la-

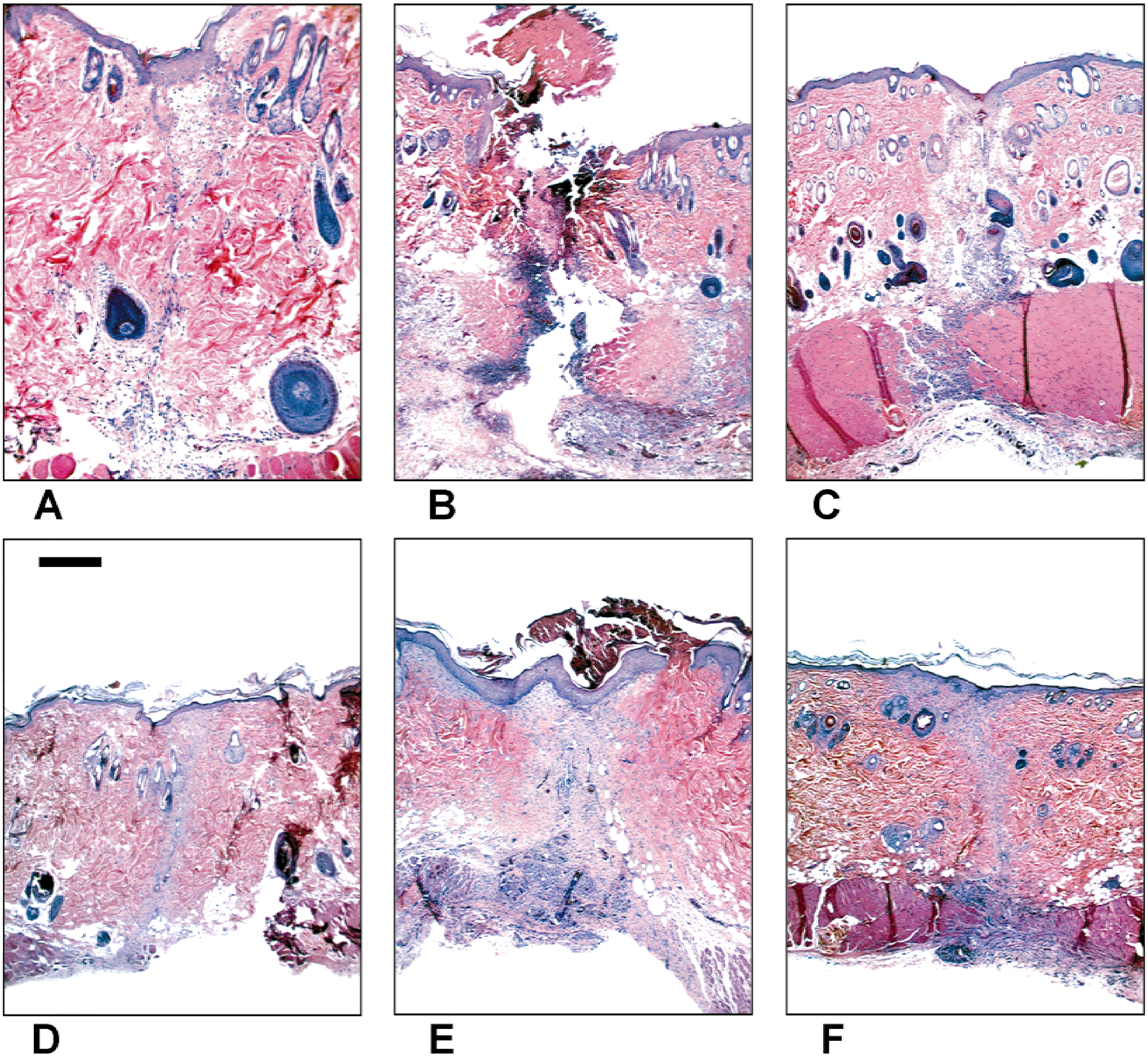

Fig. 4. Representative histology of tissue sections during wound healing. **A:** Scalpel, day 3. **B:** Continuous wave (CW) laser, day 3. Tissue separation is due to mounting artifact. **C:** Pulsed laser, day 3. Note restoration of the epithelial border. **D:** Scalpel, day 14. **E:** CW laser, day 14. **F:** Pulsed laser, day 14. For day 14, central light-staining areas indicate new collagen formation. Hematoxylin and eosin staining. Scale bar = 250 μm.

ser, as expected, produced less thermal injury when used at the 5-Hz repetition rate, averaging 118 μm of visible thermal damage, or about one-third the width of the CW laser. This value was higher than expected, considering similar studies by Walsh et al. on guinea pig skin, which found 50 μm of damage with a 2-μsec pulsed CO_2 laser [12]. Possible causes for this difference include the animal model, shorter pulse duration, or differences in staining techniques. However, the greater thermal damage we observed is not fully understood.

A thermal recovery time of 200 ms predicts an increase in thermal damage if pulses are delivered at greater than 5 Hz. Our results showed no differences in thermal injury when repetition rate was changed from 5 to 15 Hz, with a slight decrease at 10 Hz. The reason for this is uncertain, but it may be due to a number of factors. First, the differences in thermal injury between

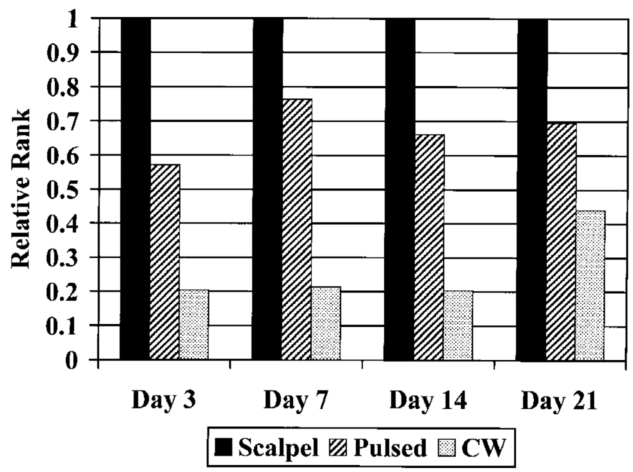

Fig. 5. Ranking of total histologic scores for healing incisions. Values are normalized by the mean scalpel rank at each time point. Values at each time point are statistically different from one another ($P < 0.05$). CW, continuous wave.

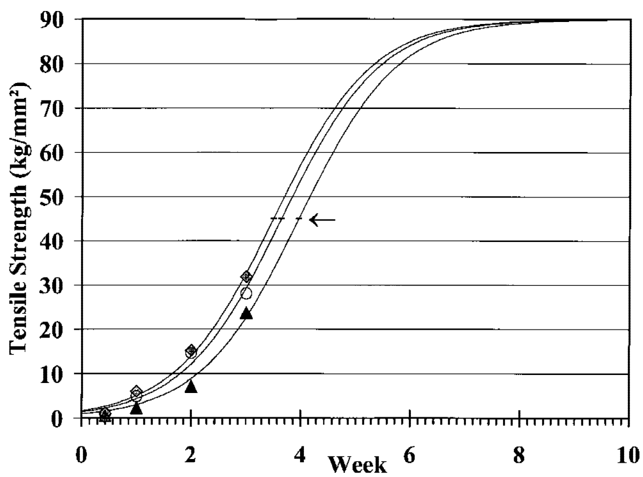

Fig. 6. Two-state mathematical model for wound tensile strength versus time; 90 kg/mm^2 at day 80 is the upper limit for the model. Solid lines represent the model for each incision type. Diamonds indicate for scalpel incisions, open circles indicate pulsed laser, and triangles indicate the continuous wave (CW) laser. The arrow and horizontal dashes indicate the point of half maximal healing and are the points used to calculate wound-healing delays in comparison with those of the scalpel. Delays were 1.0 day for pulsed laser and 3.2 days for CW laser.

repetition rate groups may be too small to distinguish for our sample size. Second, the laser was delivered by manual control via a handpiece, which could allow for variation between incisions due to human error. Third, laser delivery at 5 Hz may cause more than the minimum thermal injury due to thermal accumulation, if in fact repetition rates larger than 0.1 Hz show thermal accumulation, as proposed by Schomacker et al. [15]. Thus, increases in repetition rates to 10 and 15 Hz would cause little increase over the values measured at 5 Hz. This would be consistent with the previously described observation of a higher than expected acute thermal injury thickness at 5 Hz. The design of this experiment was to study cutting with other parameters similar to that of CW lasers currently used for surgical applications, and a reduction in pulse frequency would result in an inadequate cutting rate. Even without a complete understanding of our observed thermal damage measurements, the consistency of our measurements allows these particular delivery parameters to be evaluated for their wound-healing properties.

Any excess thermal damage beyond that accounted for by the pulse structure alone would contribute to an increase in the laser's hemostatic properties. Ideally, variation of laser repetition rate for a pulsed CO_2 surgical laser would provide a way to accurately control the thermal injury zone. This could be optimized for each tissue type or procedure to allow for a balance between cutting rate and the area of thermal damage necessary for adequate hemostasis.

Wound Healing

Both histologic and tensiometric studies showed similar results. Tensiometry showed that CW CO_2 laser incisions healed more slowly than scalpel and pulsed laser incisions in the acute and subacute time stages, but without a statistical difference in the mature wounds. This demonstrates that there is truly a "delay" in wound healing that is overcome in the later maturation phase of the wound-healing process. The decrease in thermal injury measured with pulsed laser as compared with CW would predict less of a delay in wound healing. This was in fact observed with statistically significantly stronger average breaking strengths at all time points, i.e., days 3–21. CW laser parameters were chosen from previous studies and were selected for minimal lateral thermal damage [21]. These results demonstrated a significant improvement in wound healing when using laser pulses of 7.5 μsec delivered at a frequency that produced similar cutting rates. In addition, we previously showed that more collagen is replaced when the zone of thermal damage is increased [22]. The creation of additional collagen will also add to the delay in wound healing.

The ideal surgical laser would show healing rates as fast or faster than the current standard technique, i.e., scalpel incisions. This instrument

would have all the benefits of laser incisions without the drawbacks. When we compared the wound-healing rate of the scalpel with that of the pulsed CO_2 laser, there was a trend toward slightly stronger scalpel wounds at each of the day 3–21 time points, but this difference was statistically significant only at day 21 ($P = 0.046$). In comparison, CW lasers showed much lower tensile strengths when compared with scalpel incisions. As with acute thermal injury measurements, variations in laser repetition rates had no effects on tensile strength. There were no trends or statistically significant differences across the 5-, 10-, and 15-Hz pulsed laser groups.

Our histologic results were consistent with previous descriptions of healing laser incisions [3,5,7]. Laser incisions demonstrated increased acute inflammation in areas corresponding to the zones of thermal damage. Also, the area of granulation tissue, the persistence of thermal injury at day 3 of healing, and a delay in restoration of the epithelial barrier were all dependent on the amount of acute thermally damaged tissue. These effects, representing the removal of thermally injured tissue, were present for both pulsed and CW laser incisions. Consequently, both laser groups had histology that could be distinguished from scalpel incisions, where no thermal injury was present. Scalpel histology was statistically ranked higher than either laser group. However, pulsed laser incisions had significantly higher ranking histologic scores than did CW, representing less of a barrier to wound healing. As with tensiometry, no statistical differences were observed between groups of pulsed incisions at the three repetition rates. Too few samples were studied to perform histologic ranking at the day 80 time point. However, qualitative analysis showed little difference between groups as this time, providing further evidence that laser and scalpel incisions eventually will heal with similar characteristics.

Mathematical Modeling

The goal in studying wound-healing delays is to be able to quantify the delay period. This allows for the clinical significance of these techniques to be assessed and the relevance of a new technique to be known. The two-state model is a simplification of the wound-healing processes, but it is a useful approximation. Our modeling results showed less delay, with 3.2 days by tensiometry and 6 days by histology. With incisions created with the pulsed laser, the delays were reduced to 1.0 day by both tensiometry and histology. This would indicate that CO_2 lasers cutting with short pulses (such as the 7.5-μsec pulsed width studied here) are an improvement over traditional chopped-wave CW lasers and that they demonstrate healing rates very similar to those of scalpel incisions.

CONCLUSIONS

It has been demonstrated that laser pulse duration is a key parameter in the surgical use of CO_2 lasers and that a pulse duration that is shorter than the thermal relaxation time of a tissue is able to minimize excess thermal damage when making an incision. Our study shows that this decrease in thermal damage can be correlated to an improvement in wound-healing rates. By using short pulses, such as the 7.5-μsec pulse duration used in this study, a CO_2 laser incision can be made that minimizes wound-healing delays and retains the beneficial hemostatic qualities. Our research shows that this wound-healing delay can be reduced to within 1 day of the healing rates of scalpel incisions, representing a significant improvement over CW CO_2 lasers with chopped-wave pulse durations of 0.1–0.2 sec. The use of shorter pulse durations and the minimization of thermal damage to surrounding tissue could have useful clinical applications in procedures in which CW lasers are currently the standard and possibly increase the range of applications in which a surgical laser could be used.

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